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Binding and agonist/antagonist actions of M35, galanin(1-13)-bradykinin(2-9)amide chimeric peptide, in Rin m 5F insulinoma cells

Kalev Kask, Malin Berthold, James Bourne [1], Siv Andell, Ülo Langel, Tamas Bartfai *

Department of Neurochemistry and Neurotoxicology, Arrhenius Laboratories, Stockholm University, S-106 91, Stockholm, Sweden

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Abstract

The chimeric peptide M35 [galanin(1-13)-bradykinin(2-9)amide] is a high-affinity galanin receptor ligand acting as a galanin receptor antagonist in the rat spinal cord, rat hippocampus and isolated mouse pancreatic islets. We have radiolabelled M35 and performed equilibrium binding studies with [^{125}I]M35 on the rat pancreatic β-cell line Rin m 5F, whereby we show the existence of a high-affinity binding site ($K_D = 0.9 \pm 0.1$ nM) with a B_{max} of 72 ± 3 fmol/mg protein. Galanin displaces [^{125}I]M35 with the same affinity ($K_D = 1$ nM) as it displaces [^{125}I]galanin. Displacement of [^{125}I]galanin by M35 from Rin m 5F cell membranes shows the presence of two binding sites for M35 with K_D-values of 0.3 ± 0.1 nM and 0.52 ± 0.03 μM, respectively. The GTP- and pertussis toxin-sensitivity of M35 binding to Rin m 5F membranes shows that binding of [^{125}I]M35 is almost completely abolished by the presence of GTP or after pertussis toxin treatment of the cells, indicating an agonist-like binding of M35 to the galanin receptors. M35 has a dual effect on the galanin mediated inhibition of forskolin stimulated cyclic AMP production in Rin m 5F cells: at low concentrations M35 antagonises the effect of galanin, whereas at concentrations above 10 nM M35 acts as a galanin receptor agonist. These agonist-like effects of galanin and M35 are not additive, thus the mixed agonist/antagonist properties arise from the chimeric nature of M35 [galanin(1-13)-bradykinin(2-9)amide] acting solely at galanin receptors.

Keywords: Chimeric peptide; Galanin receptor; Peptide antagonist; Cyclic AMP production; Rin m 5F insulinoma cell line

1. Introduction

Galanin, a 29 amino acid long neuropeptide [22], is widely distributed and has a number of biological functions in both the central and peripheral nervous systems as well as in the endocrine system [3,13,21]. Previously, [^{125}I]-Tyr^{26}-galanin has been used when examining the distribution of galanin receptors by equilibrium binding [9,14] and by receptor autoradiography [19]. Galanin receptors interact with the G_i/G_o subtypes of pertussis toxin ADP-ribosylable G-proteins, as shown both in ligand binding studies [9,14] and in studies of second messenger production in vitro and in vivo [6,8]. The interaction between

* Corresponding author. Fax: +46 8 161371.

[1] Present address: Department of Biochemistry, Imperial College, London, UK.

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galanin receptors and G_i/G_o-proteins has been demonstrated to be so strong that the solubilisation of rat brain membranes by (3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS) yields galanin receptor/G_i-protein α-subunit complexes [5]. In view of this strong association between galanin receptors and G_i/G_o-proteins it is desirable to characterise galanin receptors not only with agonists, but also with labelled antagonists.

We have recently designed and synthesised several chimeric peptides which act as galanin receptor antagonists in various assay systems [2]. Among these chimeric peptides, M35 – galanin(1-13)-bradykinin(2-9)amide – was shown to counteract the galanin mediated inhibition of glucose induced insulin release from isolated mouse pancreatic islets [11]. The role of endogenous galanin in the regulation of pain threshold was demonstrated using M35 as a galanin receptor antagonist [23], and furthermore, the galanin induced impairment of cognitive functions in rats can be reversed by i.c.v. injections of M35, as measured in a Morris-swim maze [24]. However, in the rat gastrointestinal smooth muscle this chimeric peptide behaves as an agonist having equal potency to galanin [12]. These data indicate that the actions of M35 at galanin receptors can be concentration-dependent and differ depending on the tissue studied and may thus reflect the presence of different subtypes of galanin receptors. In the present study, we have characterised the interactions of ^{125}I-labelled and non-labelled M35 with galanin receptors of the rat pancreatic tumour cell line Rin m 5F, which expresses a high number of galanin receptors. Radioiodination of M35 was carried out in order to directly examine ligand-receptor binding interactions, and the agonist/antagonist effect of M35 on the galanin mediated inhibition of forskolin-stimulated cAMP production was studied.

2. Materials and methods

2.1. Peptide synthesis

Peptides were assembled in a stepwise manner on a solid support using an Applied Biosystems Model 431A Peptide Synthesiser. *tert*-Boc-amino acids were coupled to 4-methyl-benzylhydryl amine resin (Bachem Feinchemikalien AG, Bubendorf, Switzerland) as hydroxybenzotriazole esters in order to obtain C-terminally amidated peptides, as described earlier [16].

2.2. Preparation of membranes of Rin m 5F cells

The establishment and cultivation of rat insulinoma Rin m 5F cell cultures has been described previously [10]. Briefly, the cells were grown in RPMI-1640 (GIBCO, Gaithersburg, MD, USA) medium containing 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin, in 4% CO_2 (v/v) enriched air at 37°C. The cells were split every 5 days. For the preparation of membrane fractions used in binding studies the cells were harvested with a rubber 'policeman' and pelleted at 400 *g* for 5 min at 4°C. The cells were exposed to hypoosmotic shock in 5 mM HEPES buffer (pH 7.4) on ice for 15 min. The suspension was centrifuged at 10,000 *g* for 15 min and the resulting pellet resuspended in bacitracin-containing (1 mg/ml) 5 mM HEPES-buffered Krebs–Ringer solution (137 mM NaCl, 2.68 mM KCl, 2.05 mM $MgCl_2$, 1.8 mM $CaCl_2$, 1 g/l glucose), pH 7.4, supplemented with 0.05% (w/v) bovine serum albumin (BSA), and used immediately in equilibrium binding experiments.

2.3. Preparation of radioiodinated galanin and M35

Porcine [^{125}I]galanin (specific activity 1800–2000 Ci/mmol), radioiodinated by the chloramine-T method using a 1 : 4 molar ratio of $Na^{125}I$ to galanin, was prepared as described before [15]. The optimal reaction conditions for the radioiodination of M35 by the chloramine-T method were preadjusted using $Na^{127}I$. For radioiodination, 2.6 nmol (5.9 μg) of M35 in 20 μl of 50 mM sodium phosphate buffer, pH 7.5, and 20 μl of chloramine-T (0.5 mM) in the same buffer were added to 5 mCi $Na^{125}I$. After 2 min, the reaction was stopped by addition of 100 μl sodium metabisulfite solution (1.2 mg/ml) and 200 μl KI solution (2 mg/ml). The reaction mixture was transferred to a SP-Sephadex C-25 column (0.8 × 10 cm) pre-equilibrated with 1 ml of 50 mM sodium phosphate buffer, pH 5.0, containing 10% (w/v) BSA and pre-eluted with 15 ml of the same buffer

without albumin. The unreacted $Na^{125}I$ was eluted with 50 mM sodium phosphate buffer, pH 5.0, and [^{125}I]M35 was eluted as a single peak with 100 mM glycine-NaOH buffer, pH 10.0. Specific activity of [^{125}I]M35 was calculated to be 500–700 Ci/mmol based on the finding that 45–47% of the peptide was iodinated by ^{127}I in 'cold' labelling to yield monoiodo[^{127}I]M35 (Fig. 1). The fractions corresponding to the radioactive peak of [^{125}I]M35 were pooled and aliquots stored at −70°C for 4 weeks without considerable loss of activity. The M35-containing solutions were collected in tubes silanised with 'Sigmacoat' (Sigma).

2.4. Ligand binding studies

Displacement experiments were performed using a filtration technique as described earlier [15], except that bacitracin (1 mg/ml) was included in the membrane preparation buffer. [^{125}I]galanin (0.2 nM) or [^{125}I]M35 (0.1 nM) were used as radiolabelled ligands. Specific binding was defined as that portion of the total binding displaceable by 1 μM unlabelled galanin.

The direct binding isotherms of [^{125}I]M35 and [^{125}I]galanin to membrane homogenates (in this case BSA was omitted from the binding buffer) were obtained by incubating the radioligand (in the concentration range from 0 to 3 nM) with the membranes at 37°C for 30 min. Specific binding at each concentration was determined and free versus bound [^{125}I]M35 or [^{125}I]galanin were plotted. Protein concentration was determined by the method of Peterson [20]. When studying the effect of GTP on M35 binding, GTP at a final concentration of 0.1 mM was added directly to the binding buffer. Pertussis toxin treatment of cells was carried out by the addition of 0.1 μg/ml of pertussis toxin (SBL, Stockholm, Sweden) to the cell culture medium for overnight incubation. Membranes were thereafter prepared as above.

All kinetic and equilibrium binding constants were calculated by fitting the experimental data using the non-linear least squares method of the program 'KaleidaGraph' on Macintosh II. K_D values for different ligands were calculated from their respective IC_{50} values using the Cheng–Prusoff equation [7].

2.5. Cyclic AMP assay

The effects of M35, galanin and bradykinin on the forskolin-stimulated cyclic AMP production were

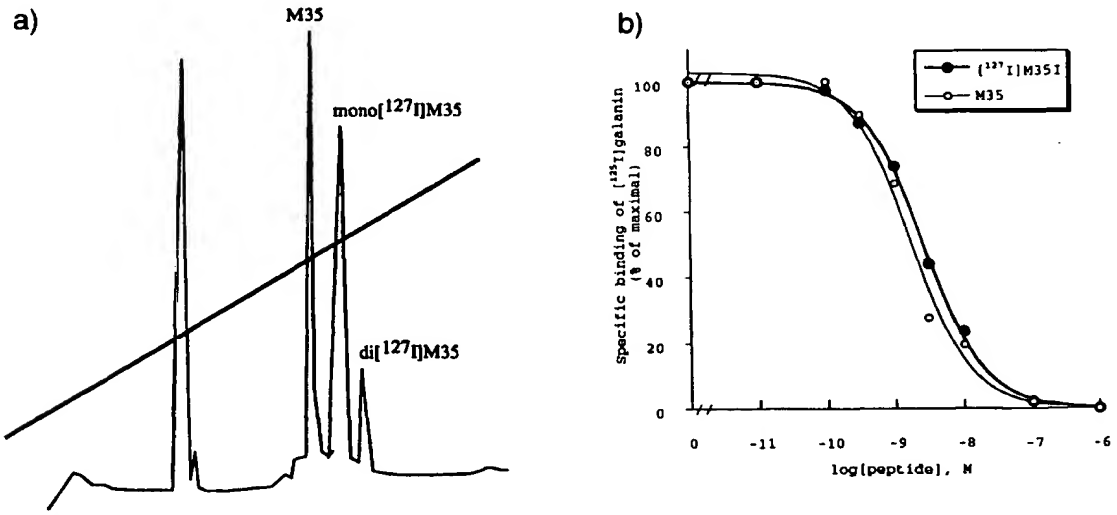

Fig. 1. (a) HPLC chromatogram showing the labelling reaction of M35 with ^{127}I. The separation was performed on a reversed-phase C-18 column using a 20–60% (v/v) gradient of acetonitrile in H_2O. The identity of peaks corresponding to non-, mono- and di-iodinated M35 was confirmed by PDMS. (b) Displacement of [^{125}I]galanin from Rin m 5F cell membranes by non-labelled M35 and [^{127}I]M35 without separation from other reaction products prior to binding.

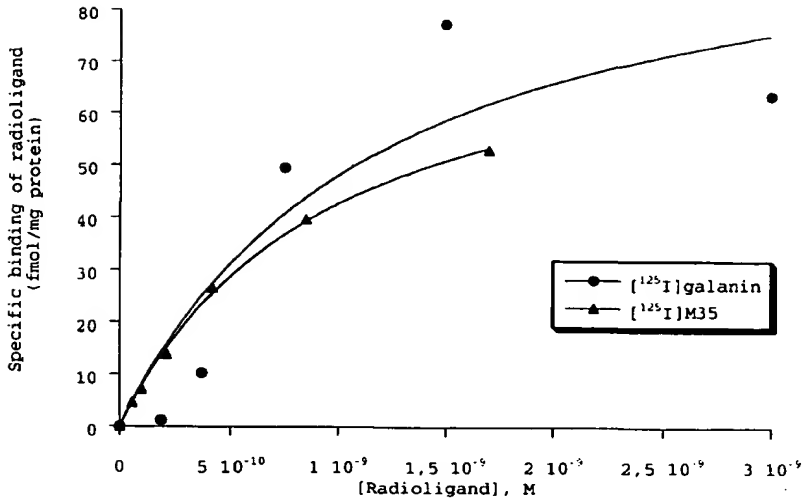

Fig. 2. Saturation binding of [^{125}I]M35 and [^{125}I]galanin to membranes of Rin m 5F cells. Non-specific binding for [^{125}I]M35 as determined in the presence of M35 (1 μM) did not exceed 45% at any concentration used of the labelled ligand. Non-specific binding for [^{125}I]galanin as determined in the presence of galanin (1 μM) did not exceed 30% at any concentration used of the labelled ligand.

studied using Rin m 5F cell membranes preincubated at 37°C for 30 min with the cyclic phosphodiesterase inhibitor IBMX (0.2 mM) and the protease inhibitors aprotinin (10 mg/ml) and bacitracin (0.5 mg/ml). Forskolin (1 μM), galanin (10 nM) and M35 (1, 5, 10, 15, 30 nM) were added to a cell suspension of 10^6 cells in a total volume of 150 μl. The incubation was carried out for 15 min at 37°C and stopped by the addition of 50 μl of EDTA (50 mM) and boiling for 3 min. The cAMP content was determined by the [^{3}H]cAMP binding assay according to Brown [4].

3. Results

M35 was radioiodinated to yield ([^{125}I]monoiodo-Tyr^9)-galanin-(1-13)-bradykinin-(2-9)amide ([^{125}I]M35). In direct saturation binding experiments

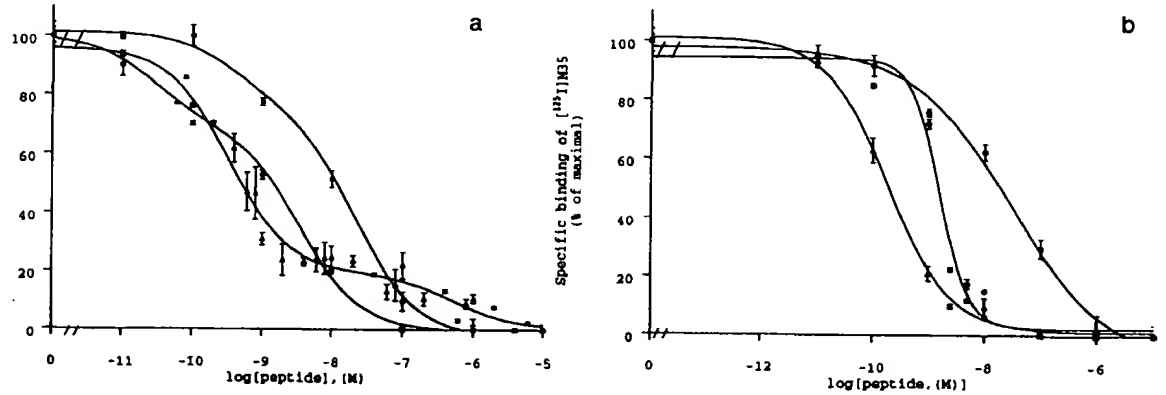

Fig. 3. Displacement of [^{125}I]galanin (a) and [^{125}I]M35 (b) from the membranes of Rin m 5F cells by M35 (triangles), galanin (dots) and galanin-(1-13) (circles). The curves represent one of two similar experiments and each point is the mean of triplicates.

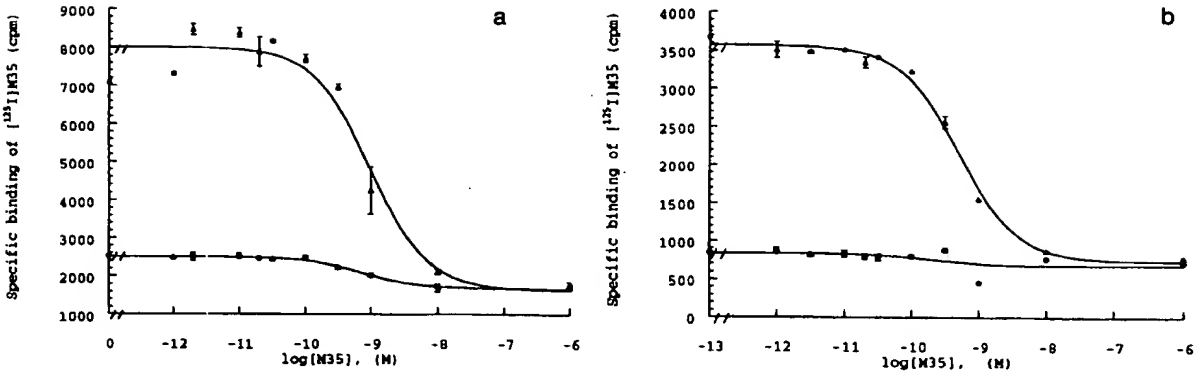

Fig. 4. Effects of GTP and pertussis toxin on the displacement of [^{125}I]M35 from Rin m 5F cell membranes by non-labelled M35: (a) (circles) GTP (0.1 mM), (triangles) control; (b) (circles) pertussis toxin (0.1 μg/ml), (triangles) control. Each curve is the representative of one experiment out of three; each point is the mean of duplicates.

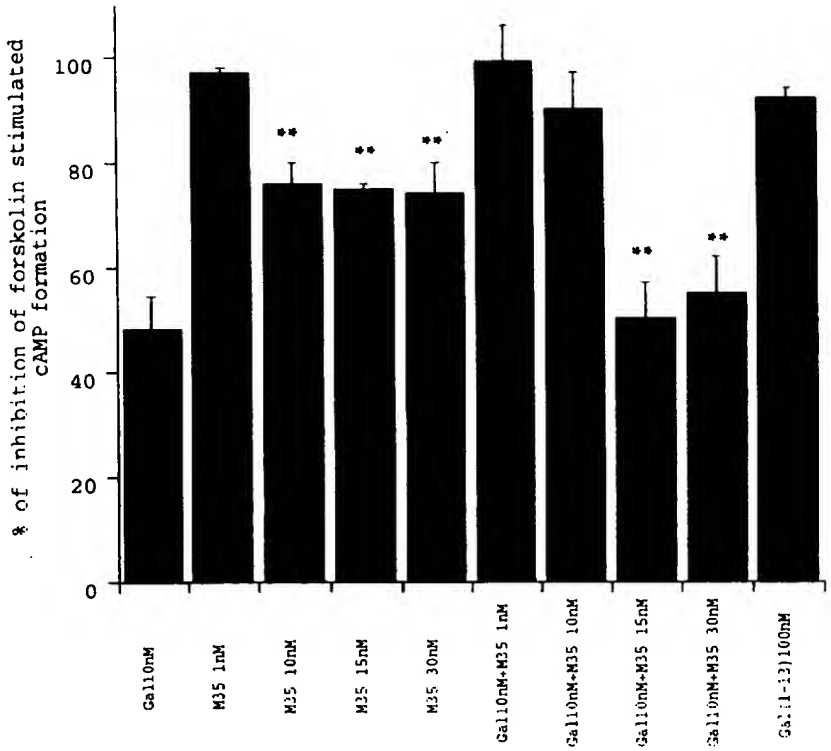

Fig. 5. The effect of galanin (10 nM) and galanin (10 nM) + M35 (1–30 nM) on the forskolin (1 μM) stimulated cyclic AMP production in Rin m 5F cells (results of one representative experiment out of five; each bar is the mean of triplicates). ** $P < 0.05$ (t-test) M35 10 nM vs. M35 1 nM; Gal 10 nM + M35 15 and 30 nM vs. M35 10 nM in the presence of 10 nM of galanin.

Table 1
Dissociation constants of M35, galanin and galanin-(1-13) in the displacement of [^{125}I]M35 and of [^{125}I]galanin from the membranes of Rin m 5F cells

Ligand	K_D(nM)	
	[^{125}I]M35 as radioligand	[^{125}I]galanin as radioligand
Galanin-(1-29)	1.5 ± 0.4	1.1 ± 0.5
M35	0.2 ± 0.1	0.3 ± 0.1 and 520 ± 30
Galanin-(1-13)	31.6 ± 15.6	19.8 ± 2.2

Data from two full displacement curves (each point is a mean of triplicates).

[^{125}I]M35 bound with high affinity ($K_D = 0.9 \pm 0.1$ nM) to a single class of binding sites on Rin m 5F cell membranes (Fig. 2). The apparent B_{max} for [^{125}I]M35 was found to be lower (72 ± 3 fmol/mg protein) than that of [^{125}I]monoiodo-Tyr^{26}-galanin, which was found to be 105 ± 16 fmol/mg protein (Fig. 2). M35 was able to fully displace [^{125}I]monoiodo-Tyr^{26}-galanin, revealing two binding sites on Rin m 5F cells with two different affinities (Fig. 3a, Table 1). The high-affinity binding ($K_D = 0.3 \pm 0.1$ nM) accounted for 77 ± 3% of the galanin binding sites, whereas the low-affinity site ($K_D = 0.52 \pm 0.03$ μM) corresponded to 19 ± 4% of the total galanin binding sites (Fig. 3a). This low-affinity site could not be assessed in direct saturation binding of [^{125}I]M35 (Fig. 2).

We could not find any data in the literature indicating the presence of any type of bradykinin receptors in this particular insulinoma cell line nor in normal pancreatic β-cells. Nevertheless, bradykinin was tested in binding experiments, but no displacement of [^{125}I]M35 by this peptide could be detected at concentrations up to 10^{-5} M (data not shown).

The effects of GTP and of pertussis toxin catalysed ADP-ribosylation on the binding of M35 to the galanin receptors of Rin m 5F cells were studied. 90% of the [^{125}I]M35 binding was abolished in the presence of GTP (0.1 mM) (Fig. 4a). Pertussis toxin pretreatment (0.1 μg/ml) of Rin m 5F cells lead to a 87% loss of the [^{125}I]M35 binding (Fig. 4b).

The effect of M35 on galanin mediated inhibition of forskolin stimulated cyclic AMP production was also studied. M35 at low concentrations (1 nM) had no effect on the forskolin stimulated production of cAMP. Galanin at 1 nM was not able to affect the forskolin stimulated cAMP production, and addition of 1 nM M35 did not alter this (data not shown). However, when co-applied with galanin (10 nM), M35 at low concentrations (1 nM) was able to reverse the inhibitory effect of galanin (Fig. 5), but when present at higher concentrations (15 and 30 nM), M35 acted as a galanin receptor agonist, inhibiting the forskolin stimulated production of cAMP. This agonist-like effect of M35 at concentrations above 10 nM was not additive to the effect of galanin at a concentration which produced maximal inhibition of the forskolin stimulated adenylate cyclase activity (10 nM) (Fig. 5). Bradykinin (1–10 μM) did not have any effect on the basal or the forskolin stimulated adenylate cyclase activity. Neither was bradykinin (10 μM) able to affect the inhibition of forskolin stimulated adenylate cyclase by galanin (10 nM) or its reversal by M35 (1 nM), respectively.

4. Discussion

The development of chimeric galanin receptor antagonists such as M15 and M35 have facilitated the elucidation of some physiological effects of endogenous galanin [1,11,18,24]. However, the mechanism on which their antagonism is based remains to be established. We show in the present study that M35 is a high-affinity galanin receptor ligand in Rin m 5F cells, recognising two binding sites with different affinities. The low-affinity binding sites ($K_D \approx$ 0.52 μM) detected by the displacement of [^{125}I]galanin by M35, are not detectable in saturation binding studies using [^{125}I]M35 (Fig. 2). These low-affinity binding sites do not arise from the interactions of M35 with bradykinin receptors albeit M35 contains bradykinin-(2-9) in its C-terminus, since bradykinin (1 μM) did not affect the binding of either [^{125}I]galanin or [^{125}I]M35.

The agonist nature of M35 is shown by the sensitivity of M35 binding to GTP and pertussis toxin catalysed ADP-ribosylation (Fig. 4). Previous studies have shown that GTP lowers the affinity of galanin to its receptors [14], similar to findings for agonists of many other G_i/G_o-protein coupled receptors. Furthermore, pertussis toxin treatment lowers the binding affinity of galanin to its receptors in rat hip-

pocampus [9] and in vivo administration of pertussis toxin abolishes the effects of galanin [8]. Here we show that the high-affinity binding of [^{125}I]M35 and of non-labelled M35 is also suppressed by the presence of GTP (0.1 mM) or by treatment of the cells with pertussis toxin (0.1 μg/ml) prior to binding studies. These effects indicate that M35 binds in an agonist manner, since antagonist binding is usually unaffected by these treatments.

The behaviour of chimeric peptides in complex biological systems is difficult to interpret, although several of these peptides have played an important part in defining the role of endogenous galanin in the peripheral nervous system [23] and in several CNS regions [1,17]. Their present use is motivated by the lack of bona fide antagonists of a peptide or non-peptide nature to the galanin receptor. We attempt here, using a simple system, to explain complex effects of chimeric peptides at galanin receptors. It is clear from this study, conducted on a homogeneous cell population possessing galanin receptors (and lacking bradykinin receptors), that the high-affinity binding of the chimeric peptide M35 is accompanied by blockade of galanin actions exerted at galanin receptors from which M35 displaces galanin. This study, however, does not adequately explain in molecular terms how the antagonist actions of M35 at low concentrations (below 10 nM) are exerted. With respect to the agonist type of actions of M35, it is clear from the lack of additivity of the effect of M35 with the effect of supra maximal concentrations of galanin that these agonist actions of M35 are also exerted at galanin receptors, and the likely reason for this is that the M35 structure contains the intact N-terminus of galanin (i.e., galanin-(1-13)) which is a low-affinity full agonist.

In conclusion, we have demonstrated here that the chimeric peptide M35, which acts as a galanin receptor antagonist in some biological systems, is a high-affinity ligand to galanin receptors of Rin m 5F cells. M35 is not a 'silent' antagonist and at higher concentrations exerts agonist action at the Rin m 5F galanin receptors inhibiting adenylate cyclase activity.

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References

[1] Bartfai, T., Bedecs, K., Land, T., Langel, Ü., Bertorelli, R., Girotti, P., Consolo, S., Xu, X. J., Wiesenfeld-Hallin, Z., Nilsson, S., Pieribone, V. A. and Hökfelt, T., M-15: high-affinity chimeric peptide that blocks the neuronal actions of galanin in the hippocampus, locus coeruleus, and spinal cord, Proc. Natl. Acad. Sci. USA, 88 (1991) 10961–10965.

[2] Bartfai, T., Fisone, G. and Langel, Ü., Galanin and galanin antagonists – molecular and biochemical perspectives, Trends Pharmacol. Sci., 13 (1992) 312–317.

[3] Bartfai, T., Hökfelt, T. and Langel, Ü., Galanin – a neuroendocrine peptide, Crit. Rev. Neurobiol., 7(3/4) (1993) 229–274.

[4] Brown, B.L., Ekins, R.P. and Albano, J.D.M., Saturation assay for cyclic AMP using endogenous binding protein, Adv. Cyc. Nuc. Res., 2 (1971) 25–40.

[5] Chen, Y., Couvineau, A., Laburthe, M. and Amiranoff, B., Solubilization and molecular characterization of active galanin receptors from rat brain, Biochemistry, 31 (1992) 2415–2422.

[6] Chen, Y., Laburthe, M. and Amiranoff, B., Galanin inhibits adenylate cyclase of rat brain membranes, Peptides, 13 (1992) 339–341.

[7] Cheng, Y.-C. and Prusoff, W.H., Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction, Biochem. Pharmacol., 22 (1973) 3099–3108.

[8] Consolo, S., Bertorelli, R., Girotti, P., La, P.C., Bartfai, T., Parenti, M. and Zambelli, M., Pertussis toxin-sensitive G-protein mediates galanin's inhibition of scopolamine-evoked acetylcholine release in vivo and carbachol-stimulated phosphoinositide turnover in rat ventral hippocampus, Neurosci. Lett., 126 (1991) 29–32.

[9] Fisone, G., Langel, Ü., Carlquist, M., Bergman, T., Hökfelt, T., Undén, A., Andell, S. and Bartfai, T., Galanin receptor and its ligands in the rat hippocampus, Eur. J. Biochem., 181 (1989) 269–276.

[10] Gazdar, A.F., Chick, W.L., Oie, H.K., Sims, H.L., King, D.L., Weir, G.C. and Lauris, V., Continuous, clonal, insulin- and somatostatin-secreting cell lines established from a transplantable rat islet tumor, Proc. Natl. Acad. Sci. USA, 77 (1980) 3519–3523.

[11] Gregersen, S., Lindskog, S., Land, T., Langel, Ü., Bartfai, T. and Ahrén, B., Blockade of galanin-induced inhibition of insulin secretion from isolated mouse islets by the non-methionine containing antagonist-M35, Eur. J. Pharmacol., 232 (1993) 35–39.

[12] Gu, Z.F., Rossowski, W.J., Coy, D.H., Pradhan, T.K. and Jensen, R.T., Chimeric galanin analogs that function as antagonists in the CNS are full agonists in gastrointestinal smooth muscle, J. Pharmacol. Exp. Ther., 266 (1993) 912–918.

[13] Kordower, J.H., Le, H.K. and Mufson, E.J., Galanin immunoreactivity in the primate central nervous system, J. Comp. Neurol., 319 (1992) 479–500.

[14] Lagny-Pourmir, I., Amiranoff, B., Lorinet, A.M., Tatemoto, K. and Laburthe, M., Characterization of galanin receptors in the insulin-secreting cell line Rin m 5F: evidence for coupling with a pertussis toxin-sensitive guanosine triphosphate regulatory protein, Endocrinology, 124 (1989) 2635–2641.

[15] Land, T., Langel, Ü., Fisone, G., Bedecs, K. and Bartfai, T., Assay for galanin receptor, Methods Neurosci., 5 (1991) 225–234.

[16] Land, T., Langel, Ü., Löw, M., Berthold, M., Undén, A. and Bartfai, T., Linear and cyclic N-terminal galanin fragments and analogs as ligands at the hypothalamic galanin receptor, Int. J. Pept. Protein Res., 38 (1991) 267–272.

[17] Leibowitz, S.F. and Kim, T., Impact of a galanin antagonist on exogenous galanin and natural patterns of fat ingestion, Brain Res., 599 (1992) 148–152.

[18] Lindskog, S., Ahrén, B., Land, T., Langel, Ü. and Bartfai, T., The novel high-affinity antagonist, galantide, blocks the galanin-mediated inhibition of glucose-induced insulin secretion, Eur. J. Pharmacol., 210 (1992) 183–188.

[19] Melander, T., Köhler, C., Nilsson, S., Hökfelt, T., Brodin, E., Theodorsson, E. and Bartfai, T., Autoradiographic quantitation and anatomical mapping of ^{125}I-galanin binding sites in the rat central nervous system, J. Chem. Neuroanat., 1 (1988) 213–233.

[20] Peterson, G.L., A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem., 83 (1977) 341–356.

[21] Skofitsch, G. and Jacobowitz, D.M., Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system, Peptides, 7 (1986) 609–613.

[22] Tatemoto, K., Rökaeus, A., Jörnvall, H., McDonald, T.J. and Mutt, V., Galanin –a novel biologically active peptide from porcine intestine, FEBS Lett., 164 (1983) 124–128.

[23] Wiesenfeld-Hallin, Z., Xu, X.J., Langel, Ü., Bedecs, K., Hökfelt, T. and Bartfai, T., Galanin-mediated control of pain: enhanced role after nerve injury, Proc. Natl. Acad. Sci. USA, 89 (1992) 3334–3337.

[24] Ögren, S.O., Hökfelt, T., Kask, K., Langel, Ü. and Bartfai, T., Evidence for a role of the neuropeptide galanin in spatial learning, Neuroscience, 51 (1992) 1–5.